π-expanded Thioxanthones – Engineering the Triplet Level of Thioxanthone-sensitizers for Lanthanide-based Luminescent Probes with Visible Excitation

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Abstract: Bright lanthanide based probes for optical bioimaging must rely on the antenna principle, where the lanthanide centred excited state is formed via a complex sensitization process. It is established that efficient sensitization of lanthanide centred emission occur via triplet states centred on the sensitizing chromophore. Here, the triplet state of thioxanthone chromophores is modulated by extending the π-system. Three thioxanthone chromophores—thioxanthone, benzo[c]thioxanthone, and naphtho[2,3-c]thioxanthone are synthesised and characterised. The triplet state energies and lifetimes is found to change as expected, and two dyes are found to be suitable sensitizers for europium(III) luminescence. Reactive derivatives of thioxanthone and benzo[c]thioxanthone are prepared and coupled to a 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A) lanthanide binding pocket. The photophysics and the performance in optical bioimaging of the resulting europium(III) complexes are investigated. It is concluded that while the energetics favour efficient sensitization, the solution structure does not. Further it was found that the complexes are too lipophilic to be efficient luminescent probes for optical bioimaging.

Introduction

To improve lanthanide-based molecular probes for bioimaging the sensitization pathway must be optimized.[1] As the most efficient sensitization pathway goes through the triplet state of a strongly absorbing chromophore,[2] the next natural step in optimizing lanthanide luminescent probes for bioimaging is triplet engineering.[3] The goal is to make bright luminescent probes that can be excited with the blue (405 nm) laser. Thus using the highest energy line in commercial microscopes, rather than the specialised costly UV laser that currently is needed to do bioimaging using lanthanide centred luminescence. This requires a narrow singlet-triplet energy splitting, and a redshift of the absorption of commonly used sensitizers above 400 nm.

Sensitized lanthanide luminescence can result in bright lanthanide luminescent probes. This is also known as the antenna principle, where the sensitizer is considered a light harvesting antenna chromophore. Direct excitation of the Ln^{3+} ions yields a low luminescence intensity due to their low molar absorption coefficients (ε ≈ 0.1 - 1 $M^{-1}cm^{-1}$).[2a, 2i] While it is possible to do optical microscopy following direct excitation,[4] bright lanthanide based probes must rely on sensitization via an antenna chromophore.[2, 5]

Triplet engineering has been done for triplet sensitizers used in photo- and polymer chemistry,[6] and in the more esoteric pursuit of excited state aromaticity.[7] One of the most explored chromophores is thioxanthone (**1**, Chart 1),[6d, 6g-j, 8] a chromophore that has also been shown to be one of the best as a sensitizer for lanthanide centred emission, in particular the coordinating azathioxanthones.[8d, 9] Methyl substitution and addition of aza-bridges does not change the photophysics of the parent thioxanthone chromophore.[10] We have previously explored the effect on the triplet state of adding electron donating groups to the azathioxanthone scaffold, see Chart 1.[10] The results confirmed that the absorption and fluorescent properties of the thioxanthone chromophore (S_1) behave as predicted by extended Hückel theory (see the supporting information for details). Unfortunately, the properties of the phosphorescent T_1 state are less easily predicted, and the addition of electron donating groups result in the desired redshift, but also unfavorable strong fluorescence.[10] Here, we chose to modulate the photophysical properties of thioxanthone by expanding the conjugated system of thioxanthone as illustrated in Chart 1. The target is absorption in the blue, a small singlet-triplet splitting, and efficient population of first the triplet state and then the lanthanide(III) centre when the chromophore is incorporated in a kinetically inert lanthanide(III) complex.[2i, 11]

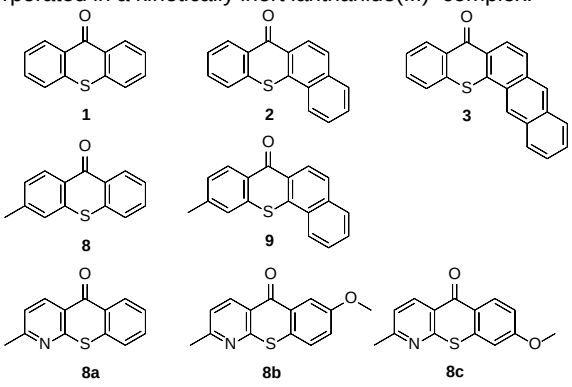

Chart 1. Model compounds thioxanthone **1**, and 3-methyl-thioxanthone **8**; π-extended thioxanthones benzo[c]thioxanthone **2**, and naphtho[2,3-c]thioxanthone **3**, and 10-methyl benzo[c]thioxanthone **9**; and 3-methyl-azathioxanthone **8a,** donor substituted 7-methoxy-3-methyl-1-azathioxanthone **8b** and 8-methoxy-3-methyl-1-azathioxanthone **8c**.

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To explore the photophysics of thioxanthones with an extended aromatic system we first synthesized the model compounds **1**, **2**, and **3** shown in Chart 1. After a full characterization of the photophysical properties of **1**-**3**, we concluded that only **1** and **2** would give rise to luminescent lanthanide complexes as the energetics of **3** were a poor match with the excited lanthanide states. Thus we synthesised DO3A ligands $\mathbf{L_1}$ and $\mathbf{L_2}$ appended with chromophore **1** and **2** respectively and prepared europium(III), terbium(III), yttrium(III) complexes these, see Chart 2. We found that the complexes have an ill-defined solution structure that resulted in inefficient sensitization of the lanthanide(III) centre. Further, the low solubility of the ligands and complexes in aqueous media resulted in less than ideal behavior in biological systems. Despite these complications, we succeeded in demonstrating that the triplet level of the thioxanthone chromophore could be engineered exclusively, and that the chromophore can sensitize europium(III) luminescence so that molecular probes based on lanthanide luminescence are possible for bioimaging following excitation at 405 nm.

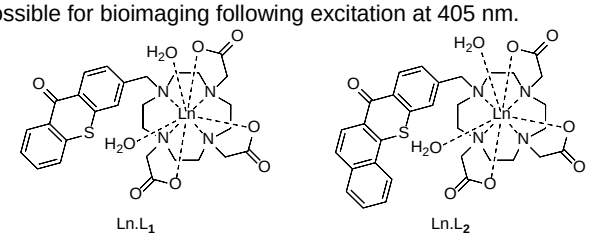


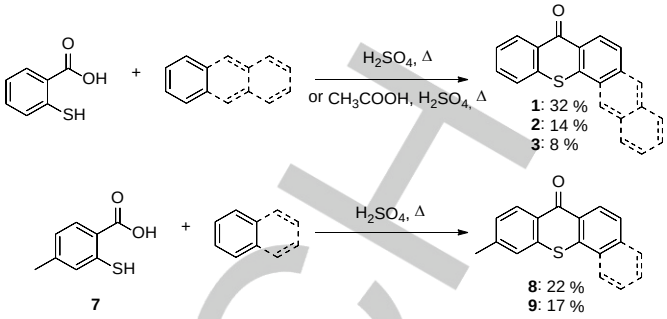
Chart 2. Lanthanide(III) complexes of thioxanthone and benzo[c]thioxanthone appended 1,4,7,10-tetraazadodecane-1,4,7-triacetic acid (DO3A) ligands $\mathbf{L_1}$ and $\mathbf{L_2}$

Results and Discussion

Synthesis

Thioxanthone **1**, benzo[c]thioxanthone **2** and naphtho[2,3-c]thioxanthone **3** were synthesised from the commercially available thiosalicylic acid and benzene, naphthalene or anthracene as outlined in scheme 1. The chosen synthetic approach was selected as it has been shown to be efficient for a variety of aromatic hydrocarbons.[12]

The two-step, one-pot synthesis of thioxanthones **1**-**3** from 2-thiobenzoic acid is shown in in Scheme 1.[13] The first step is an Ullmann type reaction yielding 2-carboxy-3'-methyldiaryl sulfide, followed by a cyclisation. The conditions shown in scheme 1 are generally applicable, yet several other combinations of starting materials and reagents have been reported.[13] The reported method of purification is recrystallization from dioxane:water,[12a-d] but to obtain analytically pure samples column chromatography (toluene:DCM or DCM, see SI) was required.[12b, 14]



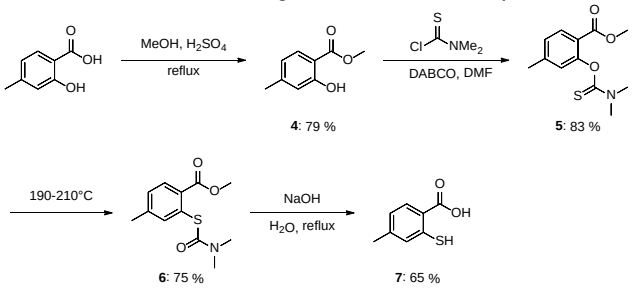
Scheme 1. Synthesis of thioxanthone **1**, benzo[c]thioxanthone **2**, naphtho[2,3-c]thioxanthone **3**, 3-methylthioxanthone **8**, and 10-methyl-benzo[c]-thioxanthone **9**.

While the mechanism for the formation of the thioxanthones from thiosalicylic acid has been investigated,[12d, 13c, 15] the constitution of the products are inconsistently reported. By extensive NMR analysis (see the SI) it was verified that compound **2** is chevron-shaped, see figure 1.

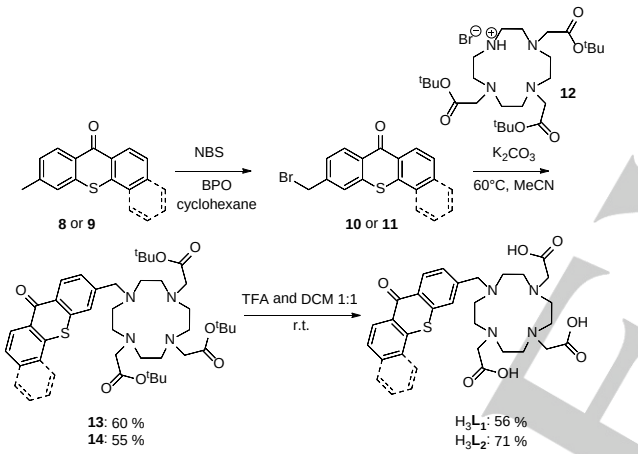
To make thioxanthones that can be linked to the 1,4,7,10-tetraazadodecane-1,4,7-triacetic acid (DO3A) lanthanide binding pocket, a methyl group is introduced in the scaffold by starting from 4-methylthiosalicylic acid **7**, see Scheme 2. To make the 4-methylthiosalicylic acid **7** a four-step synthesis starting from 4-methylsalicylic acid was used, see Scheme 2. [17] First step is protecting the carboxylic acid as the methyl ester **4**. Unreacted 4-methylsalicylic acid was removed by extraction with alkaline water and **4** was isolated as an oil in a yield of 79 %. The phenol was reacted with N,N-dimethylthiocarbamoyl chloride in the presence of DABCO that upon recrystallization gave the pure N,N-dimethylthiocarbamate ester **5** as a white powder in a 83 % yield. Following a Newman-Kwart rearrangement under an inert atmosphere of nitrogen at 190˚C **5** was converted to methyl 2-((dimethylcarbamoyl)thio)-4-methylbenzoate **6** that was isolated as an off-white product in a yield of 75 %. The Newman-Kwart rearrangement is known to give pure products.[17b] Here, the product took on a brown colour, in part due to the 20 hours required for full conversion thus, necessitating recrystallization. The final step is a double hydrolysis of **6** in alkaline water under reflux. Upon hydrolysis **7** was isolated as a white powder in 65 % yield. 3-methyl-thioxanthone **8** and the 10-methyl-benzo[c]thioxanthone **9** were synthesised from **7** using the same procedures as described for the synthesis of **1** and **2**, see Scheme 1.

In order to link thionxanthones **8** and **9** to a DO3A binding pocket a leaving group must be introduced on the α-methyl group. Wohl-Ziegler bromination of several methyl thioxanthone derivatives have previosly been reported.[13b, 18]-[19] Radical bromination was first tested in carbon tetrachloride, however, the more accessible and less toxic cyclohexane was used for the synthesis of 3-bromomethyl-thioxanthone **10** and the 10-bromomethyl-benzo[c]thioxanthone **11**. The reactant was suspended in cyclohexane with NBS. Benzoyl peroxide was used as initiator. Successive additions of NBS during the

reaction proved to give the highest yield of the final product. For **10**, the product was purified by column chromatography (DCM) and isolated as a pale yellow powder in a yield of 20 %. For **11**, column chromatography (DCM) resulted in a mixture of **11** and **9**. As only **11** will reactin the next synthetix step, the crude productwas taken forward. A yield of 8 % for compound **11** was calculated based in the integrals in the ^{1}H-NMR spectrum.



Scheme 2. Synthesis of 4-methylthiosalicylic **7**.



Scheme 3. Synthesis of thioxanthone appendend DO3A ligands $H_3\mathbf{L_1}$ and $H_3\mathbf{L_2}$.

The synthesis of ligands $H_3\mathbf{L_1}$ and $H_3\mathbf{L_2}$ were performed following a two-step synthetic route shown in scheme 3. The *tert*-butyl triester of DO3A **12** was prepared as previously described,[1b] following the procedure developed by Sammes and Faulkner.[21] Bromomethyl thioxanthone derivatives **10** or **11** was used to alkylate the secondary amine of the *tert*-butyl triester of DO3A **12** in MeCN at 60˚C with K_2CO_3. The preligands **13** and **14** were isolated as off-white and pale yellow powders in moderate yields of 60 % and 55 % after purification by column chromatography (2-5 % MeOH:DCM). Compounds **10** and **11** were used in excess (1.1 eq.), as they are easier to remove from the products compared to compound **12**.

The *tert*-butyl-ester groups of preligands **13** and **14** were removed in TFA and DCM in a 1:1 mixture at r.t.[22] The thioxanthone appended DO3A ligand $H_3\mathbf{L_1}$ and benzo[c]thioxanthone appended DO3A ligand $H_3\mathbf{L_2}$ were purified by trituration with diethyl ether from MeOH and isolated in yields of 56 % and 71 % as pale-yellow powders.

The ligands $H_3\mathbf{L_1}$ and $H_3\mathbf{L_2}$ were used to form complexes with europium(III), terbium(III), and yttrium(III). Ln.$\mathbf{L_1}$ (Ln = Y^{3+}, Eu^{3+} and Tb^{3+}) proved difficult to isolate and purify, presumably due to the low solubility. Thus, the complexes Ln.$\mathbf{L_1}$ and Ln.$\mathbf{L_2}$ (Ln = Y^{3+}, Eu^{3+} and Tb^{3+}) were prepared *in situ* from stock solutions by mixing 1:1 equivalent of $H_3\mathbf{L_1}$ or $H_3\mathbf{L_2}$ and the corresponding lanthanide(III) triflate. Ln.$\mathbf{L_1}$ and Ln.$\mathbf{L_2}$ (See chart 2) were characterised using HRMS, ^{1}H NMR and luminescence, see SI for details.

Chromophore photophysics

Even though the thioxanthone chromophore has been explored as a sensitizer for lanthanide(III) centre emission,[4c, 10, 23] and though thioxanthones with various substitution patterns have been made,[10, 24] thioxanthone derivatives with strong absorption beyond 400 nm and a small singlet-triplet gap has yet to be identified.

The photophysical properties of thioxanthones **1** and **8**, and the π-expanded thioxanthones **2**, **9**, and **3** are shown in the form of spectra in Figure 1 and the numbers are compiled in table 1. Cursory inspection of Figure 1 shows that the small singlet-triplet gap of the thioxanthones **1** and **8**, are lost in the π-expanded systems **2**, **9**, and **3**. Naphtho[2,3-c]thioxanthone **3** has a particularly large singlet-triplet splitting that makes the chromophore irrelevant for the sensitization of europium(III) and terbium(III). The reason can be found in the nature of the chromophore, where the other systems are still dominated by transitions in the thioxanthone system, the triplet state in naphtho[2,3-c]thioxanthone **3** is dominated by transitions originated in the anthracene substructure. This is evident as both the phosphorescence lifetime and the energy of the transitions are very similar to the values found for anthracene.[6c] Thus only the thioxanthones and benzo[c]thioxanthones were explored further.

Table 1. Photophysical properties in the form of absorption maximum λ_{max}, absorption cross section ε, fluorescence maximum λ_{fl}, fluorescence quantum yield φ_{fl}, fluorescence lifetime τ_{fl}, phosphorescence maximum λ_{Phos}, and fluorescence lifetime τ_{Phos} of thioxanthone **1**, benzo[c]thioxanthone **2**, naphtho[2,3-c]thioxanthone **3**, 3-methyl-thioxanthone **8**, and 10-methyl benzo[c]thioxanthone **9** in methanol.

Compound	λ_{max} nm	log(ε)	λ_{fl} nm	φ_{fl}[a]	τ_{fl} ns	λ_{Phos} nm	τ_{Phos} ms
1	378 256	3.99 4.85	434	11 %	2.8	454	161±1
8	376 258	3.84 4.77	431	12 %	3.07	450	146±6
2	387 287	3.88 4.56	440	3.2 %	0.80	512	269±5
9	385 288	3.91 4.72	434	3.0 %	0.64	507	268±1
3	426	3.80	507	1.7 %	0.71	672	24±4

	326	4.57

[a] Estimated error relative 10%.

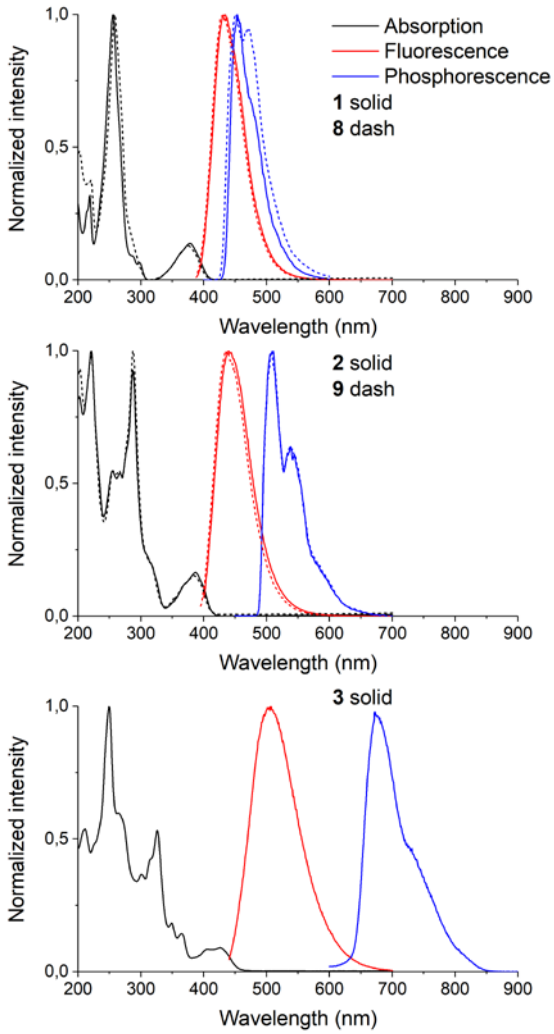


Figure 1. Absorption (black), normalised fluorescence emission spectrum (red) and normalised phosphorescence spectrum (77K, blue) of thioxanthone **1**, benzo[c]thioxanthone **2**, naphtho[2,3-c]thioxanthone **3**, 3-methyl-thioxanthone **8**, and 10-methyl benzo[c]thioxanthone **9** at 10^{-5} M in methanol.

Cursory inspection of Figure 1 show that the addition of a methyl group to the chromophore scaffold is inconsequential. Closer scrutiny of the photophysical data in Table 1 reveals that the main emissive transition occurs with similar probability ($k_f \approx 0.4 \cdot 10^8$ s^{-1}) in all the chromophores, see Table 2. So the variation in the fluorescent quantum yield from 1.7 % to 12 % (32 % if the azathioxanthones are included) is exclusively due to differences in the non-radiative rate constant k_{nr}. Table 2 includes two donor substituted azathioxanthones, **8b** with a red-shifted main absorption band and **8c** with a blue-shifted main absorption band. While the addition of donor groups in the correct positions achieve the desired redshift,[10, 23c] the result is a strongly fluorescent dye. As cursory inspection of table 2 reveals, this is not an issue with the π-extended thioxanthones that all remain poor fluorophores with similar photophysics to the parent thioxanthone chromophore. Thus we can expect that the π-extended thioxanthones are efficient sensitizers of lanthanide centred emission.

Table 2. Excited state kinetics of thioxanthone **1**, benzo[c]thioxanthone **2**, naphtho[2,3-c]thioxanthone **3**, 3-methyl-thioxanthone **8**, and 10-methyl benzo[c]thioxanthone **9** compared to those of 3-methyl-azathioxanthone **8a**, and donor substituted azathioxanthones 7-methoxy-3-methyl-1-azathioxanthone **8b** and 8-methoxy-3-methyl-1-azathioxanthone **8c**, all in methanol.

Compound	φ_{fl}[a]	τ_{fl} ns	τ_0[b] ns	k_{obs}[c] 10^8 s^{-1}	k_f[d] 10^8 s^{-1}	k_{nr}[e] 10^8 s^{-1}
1	11 %	2.8	27	3.6	0.37	3.2
8	12 %	3.07	27	3.3	0.37	2.9
2	3.2 %	0.80	25	13	0.40	12
9	3.0 %	0.64	21	16	0.48	15
8a	1.4 %	0.57	40	18	0.25	18
8b	32 %	10.4	33	1.0	0.30	0.66
8c	0.8 %	0.27	34	37	0.29	37
3	1.7 %	0.71	43	14	0.23	14

[a] Estimated relative error 10%. [b] Radiative lifetime calculated as $\tau_0 = \tau_{fl}/\varphi_{fl}$. [c] Observed rate constant for depopulation of the first excited state S_1 calculated as $k_{obs} = 1/\tau_{fl}$. [d] Rate constant of radiative deactivation of state S_1 via fluorescence $k_f = 1/\tau_0$. [e] Rate constant of non-radiative deactivation of state S_1 via fluorescence $k_{nr} = k_{obs} - k_f$.

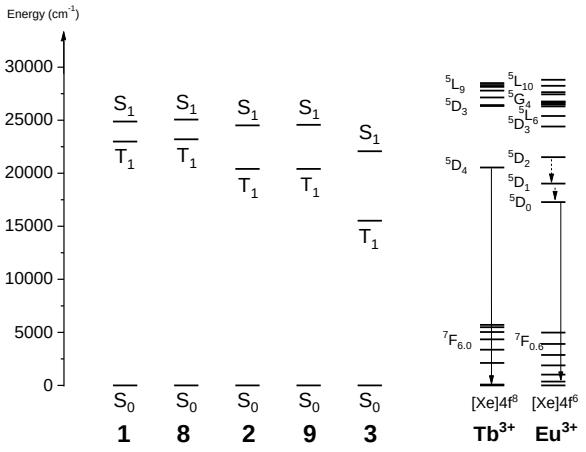


Figure 2. Energy of excited states of thioxanthone **1**, benzo[c]thioxanthone **2**, naphtho[2,3-c]thioxanthone **3**, 3-methyl-thioxanthone **8**, and 10-methyl benzo[c]thioxanthone **9** relevant in when investigating the energy transfer cascade that leads to sensitized lanthanide centred luminescence. The lowest electronic energy levels of europium(III) and terbium(III) are included.[25]

Photophysics of lanthanide complexes

Before synthesizing the thioxanthone appended lanthanide complexes we considered the relative energies of the excited states involved in the energy transfer cascade leading to lanthanide centred luminescence. As discussed above the inherent excited state kinetics of the π-extended thioxanthone chromophores should favour lanthanide sensitization if possible. The energy of the lowest singlet excited state and the lowest triplet excited state of the chromophore is plotted in Figure 2 along the lowest lanthanide(III) centred electronic states of terbium(III) and europium(III). Cursory inspection of Figure 2 shows that naphtho[2,3-c]thioxanthone **3** cannot be used as an antenna chromophore for terbium(III) or europium(III), since the excited triplet state is lower in energy than both the $^5D_4$ electronic state of Tb(III) and $^5D_0$ of Eu(III). Benzo[c]thioxanthone **2** should work only in combination with europium(III), while the parent thioxanthone **1** should work well for both europium(III) and terbium(III) when only considering energetics.[1a, 2a]

A prerequisite for efficient sensitization of lanthanide(III) luminescence is that the chromophore and the lanthanide centre are in contact.[1b, 1c, 2i, 23e] As no coordinating atoms are present in the π-extended thioxanthones, we must rely on a rigid ligand backbone to ensure that the antenna chromophore will collide with the lanthanide centre during the excited state lifetime. We recently showed that this is not the best design principle,[1c] and indeed, the ^{1}H NMR spectra in Figure 3 show that the Eu.**L_1** and Eu.**L_2** complexes have an ill-defined solution structure. This may either be adventitious, with a rapid rate of intramolecular collisions between lanthanide centre and antenna chromophore. Or a problem, if ternary complexes are formed. In these, strongly coordinating ligands bind to the lanthanide(III) centre, thus preventing the collision between the antenna chromophore and the lanthanide(III) centre.

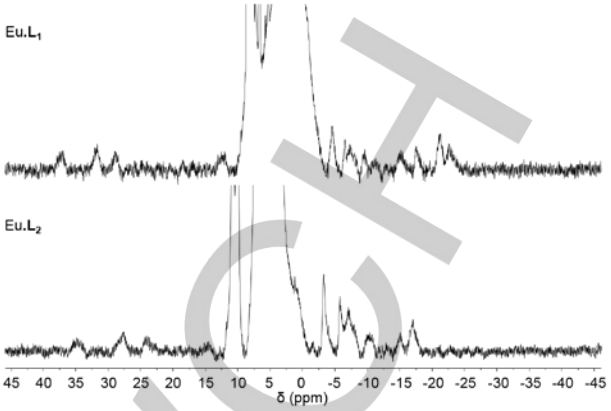


Figure 3. ^{1}H NMR spectra of Eu.**L_1** in DMSO-d6 and Eu.**L_2** in D_2O.

Figure 4 shows the excitation and emission spectra recorded in HEPES buffer at pH 7.4 for the yttrium(III), europium(III) and terbium(III) complexes of thioxanthone and benzo[c]thioxanthone appended 1,4,7,10-tetraazadodecane-1,4,7-triacetic acid (DO3A) ligands **L_1** and **L_2**. The yttrium(III) complexes are included to demonstrate that the photophysics of the chromophores are comparable in water and methanol. Cursory inspection of Figure 4 and Table 3 shows that the chromophore centred emission is similar if not identical across the complexes.

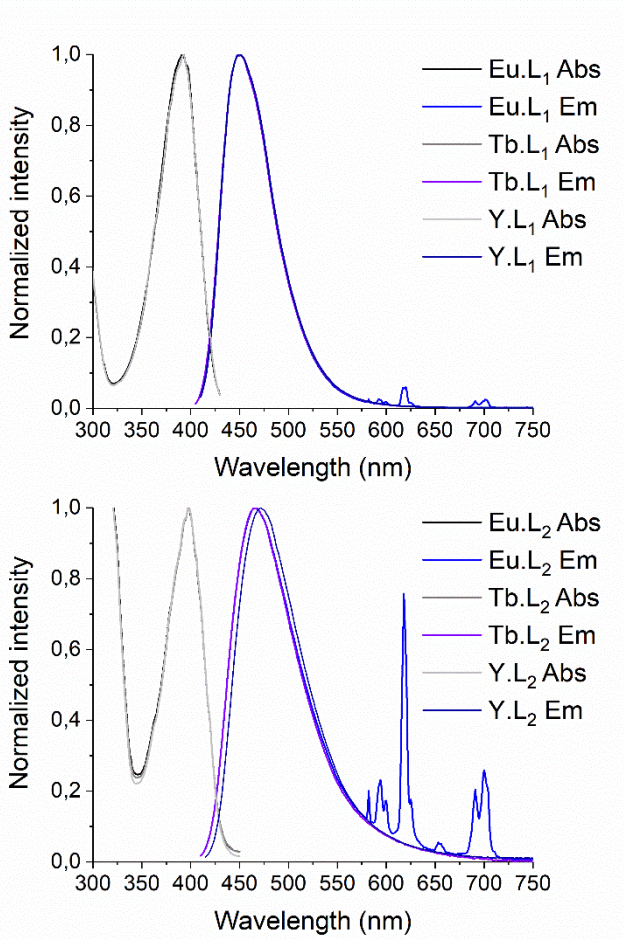


Figure 4. Top: Excitation spectra followed emission at 450 nm and emission spectra followed excitation at 392 nm of Y.**L₁**, Eu.**L₁** and Tb.**L₁**. Bottom: Excitation spectra followed emission at 465 nm and emission spectra followed excitation at 397 nm of Y.**L₂**, Eu.**L₂** and Tb.**L₂**. The spectra were recorded from 10^{-5} M solution in 0.1 pH 7.4 M HEPES buffer at ambient temperature.

While the terbium(III) complexes show no terbium(III) centred emission bands, the europium(III) complexes show both ligand centred fluorescence and bands arising from sensitized europium(III) centred emission. The excitation spectra show that all the observed emission bands originate in the chromophores centred excited state, see supporting information for details. Considering the energetics alone, Tb.**L₁** should show lanthanide centred luminescence as T_1 is higher in energy than $^5D_4$ and Tb.**L₂** should not as the ligand centred T_1 is below $^5D_4$. Clearly the population of the terbium(III) centre in Tb.**L₁** must be inefficient, despite favourable energetics.[1c] In the europium(III) complexes Eu.**L₁** and Eu.**L₂** the europium(III) centred $^5D_0$ is lower in energy than both ligand centred triplet states and europium(III) centred luminescence is indeed observed. Although the europium(III) centred band can be observed in the emission spectrum of Eu.**L₁**, they are weaker than expected. This, along with the complete lack of terbium(III) centred emission in Tb.**L₁**, indicates that the energy transfer between the thioxanthone chromophore and the lanthanide centre is inefficient in these complexes. The lanthanide centred luminescence quantum yields were determined for Eu.**L₁** and Eu.**L₂** in pH 7.4 HEPES buffer and is as expected low, see Table 3. The main reason for this is expected to be poor contact between the lanthanide(III) centre and the sensitizing chromophore. The time resolved data support this assumption as determination of *q*, the number of coordinating water molecules, show that the dominating form of the complexes has two water molecules coordinated, see Chart 2 and Table 3. This will inevitably lead to a conformation, where the sensitizing chromophore is prevented from coming in close proximity to the lanthanide centre. A small distance between antenna chromophore and lanthanide(III) centre is needed for excited state energy transfer that is sensitisation. The data show that a minor conformation with no water coordinated is also present, thus a conformation with efficient sensitization is possible but not preferred.

The conclusion must be that while the π-extended thioxanthones are chromophores with energetics that are favourable for sensitizing lanthanide(III) centred luminescence they must also include a coordinating group if they are to be used to make highly luminescent lanthanide(III) complexes. Further, the large hydrophobic π-surface makes for poorly soluble probes, this has to be circumvented by introduction of solubilizing groups. Although Eu.**L₂** has several disadvantages as a molecular probe, the complex has an absorption coefficient exceeding 10.000 $M^{-1}cm^{-1}$ at 405 nm and irreversibly populates the europium(III) centred excited state. Thus proving that π-extention of thioxanthones is a viable route to make lanthanide based molecular probes with excitation in the visible part of the spectrum

Table 3. Photophysical properties absorption maximum λ_{max}, quantum yield of sensitized europium(III) centred luminescence φ_{lum}, luminescence lifetime in $_{H2O}/D_2O$ τ, and number of inner sphere solvent molecules *q* of Y.**L₁**, Eu.**L₁** and Tb.**L₁** determined in solutions at 10^{-5} M in 0.1 pH 7.4 M HEPES buffer at ambient temperature.

	λ_{max} nm (Y.**L**)	λ_{fl} nm (Y.**L**)	φ_{lum}[a]	τ_{H2O} ms	τ_{D2O} ms	*q*[b]
Eu.**L₁**	390 (390)	450 (450)	0.02 %	1.07 (minor) 0.41 (major)	1.56	0.1 1.9
Eu.**L₂**	399 (397)	466 (471)	0.06 %	0.99 (minor) 0.41 (major)	1.48	0.1 1.8

[a] Estimated error 10%. [b] *q* calculated using:[26] $q = A(\tau_{H2O}^{-1} - \tau_{D2O}^{-1} - B))$ A: 1.2 ms; B: -0.25 ms^{-1}.

Bioimaging

Despite the low luminescence quantum yield and the low solubility of the complexes we wanted to demonstrate that the design concept is valid, and that the Eu.**L₂** complex could be used in bioimaging following excitation with a 405 nm laser. First, the binding to HSA was explored in a simple titration that showed that the complexes are solubilised by HSA and the

binding to hydrophobe compartments induce a structure of the complex that gives rise to a higher luminescence intensity, see the supporting information for details. With that in mind, NIH 3T3 cells were stained with the Eu.**L_2** complex in phenol red free cell culture medium (F12/ DMEM 1.1 supplemented with 10% FBS). The probe has demonstrated a lysosomal localisation profile, that has been verified using the commercially available stain Lysotracker Green. The localisation profile did not change with time (2 – 24h) suggesting the previously established active, predominantly macropinocitosis, uptake.[27] The probe was tested non-toxic for up to 500 uM loading at 24 hours. (can elaborate on this and send a graph if needed and a paragraph on how it has been measured). The poor photophysical properties did not afford the best conditions for imaging, but it is clearly demonstrated that the benzo[c]thioxanthone chromophore in Eu.**L_2** can be addressed using a 405 nm laser and that the resulting europium(III) centred emission can be used in bioimaging, see figure 5. So although Eu.**L_2** will never be a molecular probe, the π-extended thioxanthones does indeed afford lanthanide complexes with efficient excitation in the deep blue.

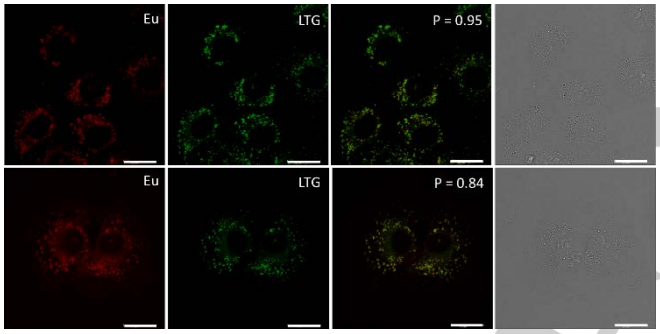


Figure 5. Laser Scanning Confocal Microscopy images of Eu.**L_2** (100 μM loading, 24 hours) using NIH 3T3 mouse skin fibroblast cell lines. (red) europium(III) centre emission (605-720 nm) recorded upon 405 nm (17 mW) excitation. (green) LysoTracker Green emission (500 – 540 nm) upon 488 nm (2 mW) laser excitation. (RGB) Merged images confirming lysosomal probe localisation with P > 0.8. Transmission images are also shown for each row confirming cell viability. Scale bars represent to 20 μm.

Conclusions

We proposed that π-extended thioxanthones would maintain the small singlet-triplet gap of the parent thioxanthone, but with red.-shifted absorption so that visible excitation can be used to excite lanthanide based molecular probes. While addition of donor and acceptor groups to the thioxanthone chromophore leads to modulation of the photophysics and both singlet and triplet energies , we found that extending the π-system exclusively leads to a modulation of the excited state energies.

Three thioxanthone dyes were synthesized and characterized in methanol. A benzo- and a naphtho-expanded thioxanthone were prepared, and both were isolated as a single isomer. The linear isomers were not isolated. π-expansion led to the desired red-shift of the singlet excited state, but also to an increased singlet-triplet gap. This to an extend that naphtho[2,3-c]thioxanthone was not able to sensitize europium(III) ions. Thus, only lanthanide complexes with thioxanthone benzo[c]thioxanthone chromophores were prepared.

The lanthanide complexes were found to have a solution structure that gave rise to poor sensitization, and consequently a low yield of lanthanide luminescence. Thus, the lanthanide complexes of the benzo-expanded thioxanthone are not bright luminescent probes, however we successfully demonstrated that they can be used in bioimaging following excitation with a 405 nm laser. This allows us to conclude that π-extension is an efficient tool for triplet engineering and that π-extended thioxanthones can be an efficient platform for making highly luminescent lanthanide complexes with visible excitation.

Experimental Section

General methods

All chemicals and solvents were used as received unless otherwise stated in the specific synthetic procedure. All chemicals purchased from Sigma-Aldrich, Merck and VWR chemicals. Both HPLC-grade and technical grade solvents were used. Cyclohexane were dried over molecular sieves (4 Å). The molecular sieves were activated by heating them to 110 degrees Celsius. Water for spectroscopy was deionised and microfiltered using a Milli-Q Millipore water purification system (resistivity > 18.2 MΩ·cm, total organic carbon (TOC) < 5 ppb). Inert atmosphere was established by using a glass bubbler with nitrogen flow. For thin layer chromatography (TLC) aluminium sheets pre-coated with silica gel 60F (Merck) was used. For column chromatography silica gel with pore size 60Å and 40-63μm or 60-200μm (for **13** and **14**) was used. Accurate masses were determined to four decimal places using a Bruker Solarix ESP-MALDI-FT-ICR instrument equipped with a 7 T magnet (the instrument was calibrated using sodium trifluoroacetate cluster ions prior to acquiring the spectrum). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 500 MHz instrument equipped with a 126 MHz (non-inverse) cryo-probe. ^{1}H NMR and ^{19}F NMR spectra were recorded on a Brucker 500 MHz instrument equipped with a broad-band-probe. All chemical shifts (δ) are given in parts per million. All NMR were recorded at 25˚C with deuterated solvents. The residual solvent was used as internal standard. Elemental analysis of the compounds was performed at the Department of Chemistry, University of Copenhagen.

9H-thioxanthen-9-one 1. Thiosalicylic acid (1.00 g, 6.54 mmol, 1 eq) was suspended in 95 % H_2SO_4 (10 ml). Benzene (2.3 mL, 25.8 mmol, 4 eq) was added dropwise over 20 min. The reaction was heated to 60 ˚C for 6 hours and left to stir at room temperature overnight. The product was precipitated from boiling water by slowly adding the reaction mixture to the boiling water (100 ml), boiled for 5 min, filtrated and washed with water. The product was dissolved in DCM, evaporated on Celite® and purified by column chromatography (DCM) to yield the product as an off-white powder after removal of the solvent under reduced pressure (0.45 g, 32 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66 – 8.60 (m, 2H), 7.65 – 7.57 (m, 4H), 7.49 (ddd, *J* = 8.2, 6.8, 1.5 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.13, 137.44, 132.42, 130.04, 129.42, 126.46, 126.14. HRMS (ESI^+): m/z for [2M+Na^+] calculated 447.0482 found 447.0482. Elem. Anal. calculated for $C_{13}H_8O_S$: C: 73.56 H: 3.80 N: -, found C: 73.43 H: 4.07 N: -.

7H-benzo[c]thioxanthen-7-one 2. Thiosalicylic acid (1.01 g, 6.55 mmol, 1 eq) was suspended in 10 ml 95 % H_2SO_4. Naphthalene (3.3 g, 25.8

mmol, 4 eq) was added over 10 min. The reaction was heated to 60 ˚C for 6 hours and left to stir at room temperature overnight. The product was precipitated from boiling water by slowly adding the reaction mixture to the boiling water (100 ml), boiled for 5 min, filtrated and washed with water. The crude material was dissolved in DCM, evaporated on Celite® and purified by column chromatography (1:3 toluene/DCM) to yield the product as a pale-yellow powder after removal of the solvent under reduced pressure (237 mg, 14 %)[a].
^{1}H NMR (500 MHz, DMSO-d_6) δ 8.52 (dd, *J* = 8.1, 1.5 Hz, 1H), 8.49 – 8.43 (m, 2H), 8.16 – 8.11 (m, 1H), 8.07 – 8.03 (m, 1H), 8.01 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.87 – 7.80 (m, 3H), 7.67 (ddd, *J* = 8.1, 7.1, 1.2 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 178.80, 136.36, 135.08, 133.89, 132.91, 129.79, 129.07, 128.77, 128.41, 128.32, 127.92, 127.42, 126.99, 126.78, 126.52, 124.04, 123.56. ^{1}H-NMR, ^{13}C-NMR, COSY, HSQC and HMBC ($CDCl_3$) spectra can be found in the SI. HRMS (ESI$^+$): m/z for $[M+H]^+$ calculated 263.0525, found 263.0527, for $[2M+Na]^+$ calculated 547.0797, found 547.0800 and for $[3M+Na]^+$ calculated 809.1249, found 809.1256. Elem. Anal. calculated for $C_{17}H_{10}OS$: C: 77.82 H: 3.84 N: -, found C: 77.61 H: 4.02 N: -.

8H-naphto[2,3-c]thioxanthen-8-one 3. Anthracene (540 mg, 3.03 mmol, 3 eq) was suspended in hot glacial CH_3COOH (10 ml). The suspension was added slowly to thiosalicylic acid (166 mg, 1.08 mmol, 1 eq). 95 % H_2SO_4 (2 ml) was added to the mixture, which was heated to reflux (118°C) for 4.5 hours. The reaction was left to stir at room temperature overnight. The product was precipitated from boiling water (150 ml). The precipitate was filtered and washed with water. The crude material was dissolved in DCM, evaporated on Celite® and purified by column chromatography (1:4 toluene/DCM). The DCM was evaporated under reduced pressure and the product was left overnight in toluene after which the product formed into crystals. The crystals were filtrated and dried over vacuum yielding the product as yellow crystals (86 mg, 8 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.18 (s, 1H), 8.76 (s, 1H), 8.54 (dd, *J* = 8.1, 1.4 Hz, 1H), 8.41 – 8.35 (m, 2H), 8.24 – 8.19 (m, 1H), 8.19 – 8.13 (m, 1H), 8.05 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.88 (ddd, *J* = 8.2, 7.1, 1.5 Hz, 1H), 7.75 – 7.64 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.49, 137.82, 135.02, 133.03, 132.88, 131.57, 130.77, 128.81, 128.75, 128.62, 127.87, 127.79, 127.64, 127.13, 127.04, 126.96, 126.68, 126.26, 123.55, 122.57. One carbon is missing. ^{1}H NMR (500 MHz, $CDCl_3$) can be found in the appendix. HRMS (ESI$^+$): m/z for $[M+H]^+$ calculated 313.0682, found 313.0690, for $[2M+Na]^+$ calculated 647.1110, found 647.1134 and for $[3M+Na]^+$ calculated 959.1719, found 959.1761. Elem. Anal. calculated for $C_{21}H_{12}OS$: C: 80.74 H: 3.87 N: -, found C: 80.72 H: 4.02 N: -.

Methyl 2-hydroxy-4-methylbenzoate 4. 4-methylsalicylic acid (10.07 g, 66.17 mmol) was dissolved in MeOH (85 ml) and 95 % H_2SO_4 (1.65 ml) was added dropwise. The reaction was refluxed for 21 hours and subsequently cooled to room temperature. The reaction was concentrated under reduced pressure and extracted with diethyl ether (3*100 ml). The organic layer was washed with aqueous $NaCO_3$ (5 % w/w) and brine, dried over magnesium sulfate and evaporated to dryness under reduced pressure yielding the product as an off-white oil (8.70 g, 79 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.70 (s, 1H), 7.70 (d, 1H), 6.79 (s, 1H), 6.69 (d, 1H), 3.92 (s, 3H), 2.33 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.68, 161.68, 147.14, 129.79, 120.56, 117.81, 109.92, 52.20, 21.96.

Methyl 2-((dimethylcarbamothioyl)oxy)-4-methylbenzoate 5. Methyl 2-hydroxy-4-methylbenzoate (**4**) (20.00 g, 120.40 mmol, 1 eq) was dissolved in DMF (40 mL). DABCO (33.83 g, 300.90 mmol, 2.5 eq) was added and the reaction mixture was stirred thoroughly for 5 min. Dimethylcarbamothioic chloride (18.65 g, 150.90 mmol, 1.25 eq) was added and the reaction mixture was heated to 70˚C for 2 hours. The reaction mixture was added slowly to a stirring mixture of ice and water. The formed precipitated was filtrated, washed with water and recrystallized from ethanol (100 ml) yielding the product as a white powder (25.24 g, 83 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, J = 8.0 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 6.96 (s, 1H), 3.84 (s, 3H), 3.49 (s, 3H), 3.41 (s, 3H), 2.43 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 187.52, 164.78, 153.66, 144.65, 131.43, 126.76, 125.38, 121.00, 51.94, 43.26, 38.86, 21.51. HRMS (ESI$^+$): m/z for $[M+H]^+$ calculated 254.0845, found 254.0847 and for $[2M+Na]^+$ calculated 529.1437, found 529.1442.

Methyl 2-((dimethylcarbamoyl)thio)-4-methylbenzoate 6. Methyl 2-((dimethylcarbamothioyl)oxy)-4-methylbenzoate (**5**) (22.36 g, 88.27 mol, 1eq) was immersed in a preheated heating mantle at 190-210˚C under an inert atmosphere of nitrogen. The reaction was left to stir for 20 hours, after which the reaction was cooled to room temperature. The crude product was recrystallized from ethanol (100 ml) yielding the product as an off-white powder (16.75 g, 75 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, *J* = 7.9 Hz, 1H), 7.47 – 7.38 (m, 1H), 7.24 – 7.19 (m, 1H), 3.86 (s, 3H), 3.07 (m, 6H), 2.38 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.04, 166.62, 142.33, 138.34, 131.97, 130.93, 129.79, 129.74, 52.25, 37.19, 37.17, 21.42. HRMS (ESI$^+$): m/z for $[M+H]^+$ calculated 254.0845, found 254.0841 and for $[2M+Na]^+$ calculated 529.1437, found 529.1433.

4-methylthiosalicylic acid 7. Methyl 2-((dimethylcarbamoyl)thio)-4-methylbenzoate (**6**) (16.75 g, 66.11 mol, 1 eq) was added to aqueous NaOH (5 M, 45 mL). The suspension was stirred for 30 min, after which the mixture was refluxed for 6 h. The reaction mixture was left to stir overnight at room temperature and then cooled to 0˚C. The pH was adjusted to 2 using aqueous HCl (6 M) and the formed precipitate was filtered. The precipitate was dissolved in NaOH (1 M) and washed with EtOAc. HCl (6 M) was added to the aqueous phase, and the formed precipitate was filtered and dried over vacuum yielding the product as a white powder (7.25 g, 65 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.1 Hz, 1H), 7.15 (s, 1H), 7.00 (d, *J* = 8.0 Hz, 1H), 4.74 (s, 1H), 2.35 (s, 3H), (One exchangeable proton from the carboxylic acid is missing). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.90, 144.41, 139.38, 132.82, 131.55, 126.06, 122.06, 21.57. Elem. Anal. calculated for $C_8H_8O_2S$: C: 57.12 H: 4.79 N: -, found C: 57.95 H: 5.48 N: -. Ikke bedre I anden omgang, jeg tror der er salt i (kan evt. bare slettes, det er kendt stof).

3-methyl-9H-thioxanthen-9-one 8. 4-methylthiosalicylic acid (**7**) (1.56 g, 5.86 mmol, 1 eq) was added to 95 % H_2SO_4 (15 ml). Benzene (2.2 mL, 24.62 mmol, 4 eq) was added dropwise over 10 min. The reaction was left stirring for 1 hour, after which the reaction was heated to 60˚C for 4 hours. The reaction mixture was slowly added to 150 ml boiling water and the aqueous solution was cooled to room temperature. The formed precipitate was filtrated and washed with water. The crude material was dissolved in DCM, evaporated onto Celite® and purified by column chromatography (DCM) to yield the product as an off-white solid after removal of the solvent under reduced pressure (454 mg, 22 %).
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 – 8.60 (m, 1H), 8.51 (d, *J* = 8.2 Hz, 1H), 7.64 – 7.55 (m, 2H), 7.48 (ddd, *J* = 8.2, 6.8, 1.4 Hz, 1H), 7.39 – 7.36 (m, 1H), 7.32 – 7.28 (m, 1H), 2.48 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.92, 143.47, 137.49, 137.38, 132.24, 129.99, 129.96, 129.50, 128.05, 127.23, 126.34, 126.12, 125.90, 21.85. COSY, HSQC and HMBC ($CDCl_3$) spectra can be found in the appendix HRMS (ESI$^+$): m/z for $[M+H]^+$ calculated 227.0525, found 227.0526, for $[2M+Na]^+$ calculated 475.0797, found 475.0798 and for $[3M+Na]^+$ calculated 701.1249, found 701.1251. Elem. Anal. calculated for $C_{14}H_{10}OS$: C: 74.31 H: 4.45 N: -, found C: 74.118 H: 4.98 N: -. Stadig ikke helt godt nok.

10-methyl-7H-benzo[c]thioxanthen-7-one 9. 4-methylthiosalicylic acid (**7**) (1.54 g, 9.16 mmol, 1 eq) was added to 95 % H_2SO_4 (25 ml). Naphthalene (4.40 g, 34.33 mmol, 4 eq) was added over 20 min to the suspension. The reaction was left stirring for 1 hours, after which the reaction was heated to 60˚C for 4 hours, and then left overnight at room

temperature. The reaction mixture was slowly added to 250 ml boiling water. The formed precipitate was filtrated and washed with water. The crude material was dissolved in DCM, evaporated onto Celite® and purified by column chromatography (1:3 toluene/DCM) to yield the product as a pale-yellow powder after removal of the solvent under reduced pressure (424 mg, 17 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.60 (d, *J* = 8.8 Hz, 1H), 8.54 (d, *J* = 8.2 Hz, 1H), 8.41 – 8.34 (m, 1H), 7.92 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.83 (d, *J* = 8.8 Hz, 1H), 7.73 – 7.61 (m, 2H), 7.49 (s, 1H), 7.33 (dd, *J* = 8.3, 1.6 Hz, 1H), 2.50 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.95, 143.21, 137.14, 136.17, 134.47, 129.62, 129.36, 129.17, 129.01, 128.54, 127.54, 127.17, 127.08, 126.46, 126.26, 125.01, 124.01, 21.86. COSY, HSQC and HMBC ($CDCl_3$) spectra can be found in the SI. HRMS (ESI$^+$): m/z for $[M+H]^+$ calculated 277.0682, found 277.0687, for $[2M+Na]^+$ calculated 575.1110, found 575.1124 and for $[3M+Na]^+$ calculated 851.1719, found 851.1748. Elem. Anal. calculated for $C_{18}H_{12}OS$: C: 78.23 H: 4.38 N: -, found C: 78.50 H: 4.37 N: -.

3-(bromomethyl)-9H-thioxanthen-9-one 10. 3-methyl-9H-thioxanthen-9-one (**8**) (306 mg, 1.35 mmol, 1 eq.) was dissolved in cyclohexane (20 ml) upon heating the reaction mixture to reflux. (The cyclohexane was dried over molecular sieves, 4 Å). NBS (120 mg, 676.11 µmol, 0.5 eq) (recrystallized from EtOH) and BPO (5 mg, 20.64 µmol, 0.2 eq) was added, and additional 0.5 eq NBS were added after 2, 4, 6, 8, 24, 26, 28, 30 and 32 hours and additional 0.2 eq BPO were added after 4, 8 and 24 hours. After 48 hours the reaction mixture was cooled to room temperature and DCM (80 ml) was added. The organic reaction mixture was washed with water, dried over magnesium sulfate and the solvent was remover under reduced pressure. The crude product was purified by column chromatography (DCM) yielding the product as a yellow solid (85 mg, 20 %) after removal of the solvent, in addition 140 mg (46 %) of starting material was recovered. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 – 8.57 (m, 2H), 7.66 – 7.55 (m, 3H), 7.53 – 7.46 (m, 2H), 4.54 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.58, 142.28, 137.88, 137.18, 132.57, 130.70, 130.05, 129.40, 129.09, 127.19, 126.67, 126.26, 126.17, 31.72. HRMS (ESI$^+$): m/z for $[M+H]^+$ calculated 304.9630, found 304.96335 and for $[2M+Na]^+$ calculated 632.8992, found 632.8981.

10-(bromomethyl)-7H-benzo[c]thioxanthen-7-one 11. 10-methyl-7H-benzo[c]thioxanthen-7-one (**9**) (300 mg, 1.09 mmol, 1 eq.) was dissolved in cyclohexane (25 ml) upon heating the reaction mixture to reflux. (The cyclohexane was dried over molecular sieves, 4 Å). NBS (100 mg, 561.85 µmol, 0.5 eq) (recrystallized from EtOH) and BPO (10 mg, 43.42 µmol, 0.04 eq) was added, and additional 0.5 eq NBS were added after 2, 4, 6, 8, 24, 26, 28, 30 and 32 hours and additional 0.04 eq BPO were added after 8 and 24 hours. After 48 hours the reaction mixture was cooled to room temperature and DCM (100 ml) was added. The organic reaction mixture was washed with water, dried over magnesium sulfate and the solvent was remover under reduced pressure. The solid obtained was purified by column chromatography (DCM) yielding the crude product as a yellow powder (60 mg, purity 53 % corresponding to a yield of 31 mg, 8 %) after removal of the solvent. Additional starting material was recovered (154 mg, 51 %). The product was used in the next synthesis step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (d, *J* = 8.3 Hz, 1H), 8.63 (d, *J* = 8.8 Hz, 1H), 8.44 (m, 1H), 8.00 – 7.96 (m, 1H), 7.90 (dd, *J* = 8.9, 0.8 Hz, 1H), 7.78 – 7.68 (m, 3H), 7.58 (dd, *J* = 8.3, 1.8 Hz, 1H), 4.61 (s, 2H).* HRMS (ESI$^+$): m/z for $[M+H]^+$ calculated 354.9787, found 354.9788. *A small fraction pure product from the column chromatography was collected for ^{1}H NMR.

1,4,7-tri(tert-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, hydrobromide salt 12.[1b, 21] Cyclen (5.01 g, 29.08 mmol, 1 eq) was dissolved in acetonitrile (120 ml). Sodium bicarbonate (8.09 g, 96.30 mmol, 3.3 eq) was added to the solution. The suspension was stirred vigorously for 30 min while cooled using an ice bath. Tert-butyl bromoacetate (14.20 ml, 96.10 mmol, 3.3 eq) was added dropwise over 35 min and the reaction was left to stir at room temperature for 72 hours. The formed inorganic solid was removed by filtration and the solvent was removed under reduced pressure. The crude beige solid was recrystallized four times from hot toluene yielding the product as a white solid (4.34 g, 25 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.04 (s, 2H), 3.37 (s, 4H), 3.29 (s, 2H), 3.16 – 3.04 (m, 4H), 3.01 – 2.80 (m, 12H), 1.46 – 1.45 (m, 25 H) (Two exchangeable protons are missing). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.66, 169.77, 82.00, 81.85, 58.39, 51.53, 49.37, 47.68, 28.39, 28.35. Two quaternary carbon are missing. HRMS (ESI$^+$): m/z for $[M+H]^+$ calculated 515.3803, found 515.3798.

1-(3-methyl-9H-thioxanthen-9-onyl)-4,7,10-tris(tert-butoxycarboxylmethyl)-1,4,7,10-tetraazadodecane 13. 3-(bromomethyl)-9H-thioxanthen-9-one (**10**) (46 mg, 151 µmol, 1.06 eq), 1,4,7-tri(tert-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, hydrobromide salt (**12**) (85 mg, 143 µmol, 1 eq) and K_2CO_3 (85 mg) was added to MeCN (5 mL) and heated to 60˚C overnight. After 16 hours the reaction mixture was cooled to room temperature and the inorganic solid was removed by filtration. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (5 % MeOH/DCM) to yield the off-white solid product after removal of the solvent under reduced pressure (67 mg, 60 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 – 8.41 (m, 2H), 7.82 (d, *J* = 1.4 Hz, 1H), 7.72 – 7.41 (m, 4H), 3.81 – 1.94 (m, 44H), 1.71 – 1.13 (m, 27H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.67, 173.82, 172.84, 143.28, 137.99, 137.29, 132.56, 130.29, 129.99, 129.36, 128.55, 127.91, 127.68, 126.63, 126.16, 83.06, 82.41, 59.82, 56.24, 55.92, 53.57, 28.03. HRMS (ESI$^+$): m/z for $[M+H]^+$ calculated 739.4099, found 739.4161, and for $[M+Na]^+$ calculated 761.3924, found 761.4000.

1-(10-methyl-7H-benzo[c]thioxanthen-7-onyl)-4,7,10-tris(tert-butoxycarboxylmethyl)-1,4,7,10-tetraazadodecane 14. 10-(bromomethyl)-7H-benzo[c]thioxanthen-7-one (**11**) (210 mg, 50 % purity, corresponding to: 105 mg, 295 µmol, 1.1 eq), 1,4,7-tri(tert-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, hydrobromide salt (**12**) (156 mg, 261 µmol, 1 eq) and K_2CO_3 (50 mg) was added to MeCN (15 mL) and left to stir at 60˚C overnight. The reaction mixture was cooled to room temperature and the inorganic solid was removed by filtration. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (2 % MeOH/DCM to remove impurities and excess of starting material and 5 % MeOH/DCM to elute the product). The product was obtained as a pale-yellow powder after removal of the solvent under reduced pressure (114 mg, 55 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 – 8.50 (m, 2H), 8.37 (dd, J = 8.4, 1.1 Hz, 1H), 8.14 – 7.83 (m, 3H), 7.83 – 7.53 (m, 3H), 4.90 – 1.74 (m, 24H), 1.70 – 1.16 (m, 27H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.76, 173.83, 172.86, 143.17, 137.17, 136.66, 134.57, 130.00, 129.49, 129.29, 129.17, 128.52, 128.44, 128.11, 127.55, 127.32, 126.85, 124.90, 123.78, 83.07, 82.43, 59.81, 56.25, 55.87, 28.03. HRMS (ESI$^+$): m/z for $[M+H]^+$ calculated 789.4255, found 789.42768, and for $[M+Na]^+$ calculated 811.4075, found 811.41145.

1-(3-methyl-9H-thioxanthen-9-onyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazadodecane H_3L_1. 1-(3-methyl-9H-thioxanthen-9-onyl)-4,7,10-tris(tert-butoxycarboxylmethyl)-1,4,7,10-tetraazadodecane (**13**) (97 mg, 131.26 µmol) was dissolved in DCM (5 mL), and TFA (5 mL) was added dropwise to the solution. The reaction was stirred at room temperature for 48 hours. The solvent was removed under reduced pressure and the remaining oil was dissolved in a minimum amount of MeOH and precipitated with diethyl ether. Trituration with diethyl ether from methanol yielded the product as a pale-yellow powder (42 mg, 56 %). ^{1}H NMR (500 MHz, D_2O) δ 8.33 (d, *J* = 8.1 Hz, 1H), 8.24 (d, *J* = 8.2 Hz, 1H), 7.72 – 7.43 (m, 5H), 3.75 (s, 2H), 3.65 – 3.25 (m, 14H), 3.17

(s, 4H), 2.93 (s, 4H). pH ~ 11. HRMS (ESI^+): m/z for $[M+H]^+$ calculated 571.2221, found 571.2224.

1-(10-methyl-7H-benzo[c]thioxanthen-7-onyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazadodecane H_3L_2. 1-(10-methyl-7H-benzo[c]thioxanthen-7-onyl)-4,7,10-tris(tert-butoxycarboxylmethyl)-1,4,7,10-tetraazadodecane (**14**) (100 mg, 126.74 µmol) was dissolved in DCM (5 mL), and TFA (5 mL) was added dropwise to the solution. The reaction was stirred at room temperature for 24 hours. The solvent was removed under reduced pressure and the remaining oil was dissolved in a minimum amount of MeOH and precipitated with diethyl ether. Trituration with diethyl ether from methanol yielded the product as a pale-yellow powder (56 mg, 71 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.56 – 8.42 (m, 3H), 8.21 – 8.12 (m, 1H), 8.12 – 8.04 (m, 2H), 7.85 (p, J = 7.1 Hz, 2H), 7.73 (d, J = 8.3 Hz, 2H), 4.20 (s, 2H). Additional peaks: 3.67, 3.56, 3.17, 3.13, 2.98 (not integrated due to the water signal). HRMS (ESI^+): m/z for $[M+H]^+$ calculated 621.2377, found 621.2376.

LnL_1 and LnL_2 (Ln= Y^{3+}, Eu^{3+} and Tb^{3+}). For the photophysical measurements the complexes Ln.**L_1** and Ln.**L_2** (Ln= Y^{3+}, Eu^{3+} and Tb^{3+}) were assembled *in situ* from aqueous stock solutions by mixing 1:1 equivalent of the H_3**L_1** or H_3**L_2** and $Ln(OTf)_3$ (Ln= Y^{3+}, Eu^{3+} and Tb^{3+}) in aqueous 0.1 M HEPES buffer at pH 7.4 (3 mL), such that concentration of the ligand and the Ln^{3+} ion were 10^{-5} M. The solutions were left at room temperature for at least 48 hours to let the complexation go to completion.
To obtain ^{1}H-NMR spectra, the complex Eu.**L_1** was prepared *in situ* by mixing 1:1 equivalents of H_3**L_1** and $Eu(OTf)_3$ in DMSO-d_6. Eu.**L_2** was prepared similarly *in situ* by mixing 1:1 equivalents of H_3**L_2** and $Eu(OTf)_3$ in D_2O. The complexes were left to stir at 60˚C for at least 48 hours. Both reactions were tried in deuterated water, methanol and DMSO, however due to solubility issues it was only possible to obtain a ^{1}H-NMR spectrum from the DMSO-d_6 for Eu.**L_1** and D_2O for Eu.**L_2**.

Optical spectroscopy

For all measurements, the absorbance at the excitation and at longer wavelengths was kept below 0.1 to avoid inner filter effects. All fluorescence and lanthanide luminescence experiments were performed at ambient temperatures in Hellma or Starna Quartz cuvettes with 10 mm path length and all phosphorescence experiments were performed at 77 K in a quartz tube.

Absorption spectra were measured with a Cary 300 UV/Vis double beam spectrometer from Agilent Technologies against air using a pure solvent baseline.

The fluorescence, phosphorescence and time-gated lanthanide luminescence spectra were recorded on a Cary Eclipse fluorescence spectrometer with a photomultiplier tube from Agilent Technologies. Phosphorescence lifetimes were measured on the same instrument and fitted as monoexponential decays using the Origin software package. The phosphorescence experiments at 77 K were performed with a quartz cold finger accessory from PTI (WG_850_Q_PTI); the sample solution was placed in a quartz tube from Mirit Glas and flash frozen using liquid nitrogen.

The fluorescence and lanthanide luminescence lifetimes were determined using a FluoTime 300 instrument from PicoQuant. The excitation source was the 355 nm line from a VisUV laser or a Xenon flash lamp from PicoQuant. The data was fitted using the deconvolution as implemented in the FluoFit software (version 4.6.6) from PicoQuant.

Steady-state spectra of the lanthanide luminescence were recorded on a PTI QuantaMaster 8075 spectrofluorometer from Horiba Scientific with a 75W Xenon Arc Lamp from Horiba Scientific. Emission corrected using manufacturer supplied detector sensitivity correction file and excitation corrected using a RCQC photodiode and manufacturer supplied correction file.

Quantum yield determination

For all measurements the absorbance at the excitation and at longer wavelengths were kept below 0.1 to avoid inner filter effects. All measurements were perfomed in 10 mm quartz cuvettes from Starna Scientific. Quantum yields were determined using IUPAC recommended multipoint determination using Quinine sulfate as a reference (Φ_{fl} = 0.546)[28].

$$\phi_{fl} = \phi_{fl}(ref) \cdot \frac{A(ref)}{A} \cdot \frac{\eta^2}{\eta^2(ref)} \frac{\int I_{fl}}{\int I_{fl}(ref)} \qquad (1)$$

where Φ_{fl} is the fluorescence quantum yield, A is the absorption at the excitation wavelength, η is the refractive index and ∫I is the integrated emission intensity.

Absorption measurements were measured on a Cary 300 UV/Vis double beam spectrometer from Agilent Technologies against air using pure solvent as a reference. Slits were kept at 2 nm.

Emission spectra were recorded on a PTI QuantaMaster 8075 from Horiba Scientific with a 75W Xenon Arc Lamp from Horiba Scientific at room temperature. Emission corrected using manufacturer supplied detector sensitivity correction file and excitation corrected using a RCQC photodiode and manufacturer supplied correction file. Excitation and emission slits were kept at 8 and 2 nm respectively. The difference in slit width for absorption and emission spectra does not influence the quantum yields due to the broad nature of the excitation band. Excitation was done at 370 nm.

All spectra used in the determination can be found in the supporting information.

Cell imaging

High resolution Laser Scanning Confocal Microscopy (LSCM) images were recorded on a modified Leica SP5 II microscope, equipped with a new SIM technique called PhMoNa.[Pal faraday paper if we cna if not no worries) In order to achieve excitation with maximal probe emission, the microscope was equiped with a 405 nm diode laser, operating at 17 mW power. A He/Ne or Ar ion laser was used when commercially available organelle-specific stains (e.g. LysoTrackerGreenTM, 488 nm excitation, emission 500 – 540 nm) were used to corroborate cellular compartmentalization.

The microscope was equipped with a triple channel imaging detector, comprising two conventional PMT systems and a HyD hybrid avalanche photodiode detector. The latter part of the detection system, when operated in the BrightRed mode, is capable of improving imaging sensitivity above 550 nm by 25%, reducing signal to noise by a factor of 5. The pinhole was always determined by the Airy disc size, calculated from the objective in use (HCX PL APO 63x/1.40 NA αBlue), using the lowest excitation wavelength (405 nm). Scanning speed was adjusted to 100 Hz in a unidirectional mode, to ensure both sufficient light exposure and enough time to collect the emitted light from the lanthanide based

optical probes (1024 x 1024 frame size, a pixel size of 96 x 96 nm and depth of 770 nm)

Live cell culture studies

A detailed investigation of the cellular behavior of each complex was conducted using NIH 3T3 mouse skin fibroblast cell using epi-fluorescence and laser scanning confocal microscopy. Initial cell line was sourced from ATCC (NIH 3T3 CRL-1658) and have been established and maintained in a category 2 cell culture facility according to established standardized protocol for 12 months; they have been periodically monitored for mycoplasma contamination. Cells were maintained in exponential growth as monolayers in F- 12/DMEM (Dulbecco's Modified Eagle Medium) 1:1 that was supplemented with 10% fetal bovine serum (FBS) or human or goat serum where appropriate. Cells were grown in 75 cm2 plastic culture flasks, with no prior surface treatment. Cultures were incubated at 37 °C, 10% average humidity and 5% (v/v) CO2. Cells were harvested by treatment with 0.25% (v/v) trypsin solution for 5 min at 37 ºC. Cell suspensions were pelleted by centrifugation at 1000 rpm for 3 min, and were re-suspended in fresh medium by repeated aspiration with a sterile plastic pipette. Microscopy cells were seeded in untreated iBibi 100 µL live cell channels and allowed to grow to 40% to 60% confluence, at 37 ºC in 5% CO2. At this stage, the medium was replaced and cells were treated with the studied Eu-complex and co-stains as appropriate, present in the final imaging medium. For live cell imaging, DMEM/F12 media lacking phenol red was used from this point onwards using a purpose build incubator housing the microscope maintaining 37 ºC, 5% CO2 and 10% humidity.

The europium complexes (10 µM) were incubated with NIH-3T3 cells for up to 24h to allow complex uptake within the lysosomes; this localisation profile was verified by co-incubation with LysoTracker-Green.

Cell toxicity test

Cell toxicity was determined using a ChemoMetec A/S NucleoCounter3000-Flexicyte instrument with Via1-cassette cell viability cartridge using the cell stain Acridine Orange for cell detection, and the nucleic acid stain DAPI for detecting non-viable cells and Annexin V for the detection of apoptosis. In cellular uptake studies, cells were seeded in 6-well plates and allowed to grow to 80% to 100% confluence at 37 ºC in 5% CO2. Culture medium was then replaced with culture medium containing the probe at 8 concnetration points in the range of 1-500 uM for 24 h. All cell colonies bearing EuL3 displayed 92 ± 5% viability; the control blank cells were established at 95 ± 3% viability.

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π-expanded Thioxanthones – Engineering the Triplet Level of Thioxanthone-sensitizers for Lanthanide-based Luminescent Probes with Visible Excitation